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Experimental Immunization of Man Against Botulism

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Numerous investigations have been conducted in the field work of immunity against botulism. They involved many trends and a great number of animal species. The results of these experiments showed, pertinently to the use of anatoxin for effective immunization, that animals can develop immunity of considerable potency. In many instances even a single injection of anatoxin produced in a guinea pig the immunity of sufficient strength against 1 million fatal doses of botulinal toxin (VELIKANOV). Thus, we became interested in a preparation of antigenic material against botulism in man and we subjected human beings to immunization with anatoxin.

Having formulated a problem to develop an effective antigen against botulism in man, we subsequently performed essential tests using normal sera of these persons.

The obtained sera were diluted to 1:10 and 1:2, but we also used whole sera, and the quantity of toxin comprised 10 fatal doses. While performing the tests with normal sera (obtained from 9 persons between 26 and 48 years old), we invariably obtained negative results. Hence, a normal human serum does not hold antitoxin against botulism.

Having completed this investigation, we proceeded to immunize:

human beings. We used for antigen the anatoxin obtained from the type A and B toxins, which were processed with formalin at  $37^{\circ}\text{C}$  for 30 days. Prior to administration of antigen, we carefully tested the latter as to its harmlessness, injecting it to a guinea pig ( $10\text{ cm}^3$ ) and to a mouse ( $2\text{ cm}^3$ ).

Our first experiment began on 2 volunteers, and their anatoxin dose was  $0.5\text{ cm}^3$  in the first injection. In applying the anatoxin hypodermic injection we tried not to inflict pain. Then, we made careful medical observations on inoculated volunteers for 10 days, but we failed to find any complicated symptoms: the organism showed neither localized nor general pathogenic symptoms after the inoculation.

This enabled us to proceed with the second injection in the same dose, which was given after 11 days following the first one. Like the first one, also this injection was hypodermic. Neither personal complaints, nor complications resulted from the second experiment. Hence, a correctly prepared anatoxin of botulism appeared harmless to man.

With the expiration of 11 days after the two injections, we decided to examine the blood serum of immunized testees as to the presence of antitoxin. The serum was diluted 1:10 and 1:50. The titration test of serum so diluted and containing 10 fatal doses of the toxin produced negative results. Thus, the two anatoxic injections of  $0.5\text{ cm}^3$  each left no appreciable immunization stimulation in the organism.

Consequently, we prolonged the immunization of the two testees.

We administered the third injection of the same anatoxin, in the same dose of 0.5 cm<sup>3</sup>. Also this injection passed safely, and neither localized, nor general complications resulted.

We took another blood test on the 16th day after the third injection and the obtained serum was examined as to the presence of botulinal antitoxin. The result was negative.

Then, taking advantage of our old records referring to the presence of the maximal strength of immunity in guinea pigs on the 30th to 35th day, we decided to verify the condition of the serum taken at a much later date. The blood was taken on the 35th day after the third injection, and thus obtained serum in dilution 1:2 and mixed with 10 fatal doses of the type A toxin was subjected to a test. Again the results were negative.

The experiment performed on the two testees permits us to draw the following conclusions: 1) the anatoxin of Cl. botulinum type A and B, accurately prepared, is absolutely harmless to human beings; 2) a threefold subcutaneous injection of 0.5 cm<sup>3</sup> each failed to produce general and local symptoms; 3) a threefold vaccination of 2 testees with anatoxin type A in 0.5 cm<sup>3</sup> dose each provided no appreciable immunization stimulation. Sera diluted 1:2 were incapable of neutralization of 10 fatal doses of appropriate toxin.

Having obtained these results, we had a right to expand our experiments, i.e. to conduct them on a greater number of volunteers and to increase the doses of administered antigen.

A new group of testees received 5 subcutaneous injections each: the first two injections of 0.5 cm<sup>3</sup> each and the next three inject-

ions of 1 cm<sup>3</sup> each. Consequently, each testee received 4 cm<sup>3</sup> of anatoxin. The injections were administered with time intervals of 10 to 12 days. We received no complaints as to unpleasant feelings

Table 1

Name	Dilution of serum	Lethal doses of toxin	Results	Weight of guinea pig
P	1:2	2	Alive	350
P	1:5	2	Died after 2 days	350
Control	—	2	Died after 2 days	350

at the time of injections. We observed neither complications, nor local and general reactions resulting from administration of anatoxin.

We tested the sera of vaccinated people twice as to the presence of anatoxin. The first blood test was taken after 12 days following the 4th injection.

This experiment produced no definite results, and the only effect that could be mentioned is that we observed in guinea pigs some tendency toward a delay of their time of death after they received a mixture of the toxin and serum.

The serum for the second test was taken on the 12th day after the 5th injection (see Table 1).

This experiment brought a satisfactory result: the serum diluted 1:2, taken for testing after 12 days following the 5th injection, protected a guinea pig from 2 fatal doses of the toxin. Consequently, a fivefold immunization of man with anatoxin caused

a development of antitoxin in his blood.

Table 2

Name	Age	Dilution of serum	Lethal doses of toxin	Results	Time of taken serum
B	68	1:2	2	Died after 12 days	After 10 days following 4th injection
		1:5	2	Died after 4 days	" " " "
P	70	1:2	2	Died after 5 days	After 23 days following 4th injection
		1:5	2	Died after 4 days	" " " "
X	53	1:2	2	Alive	After 23 days following 3rd injection
		1:5	2	"	" " " "
M	65	1:2	2	Died after 5 days	After 23 days following 4th injection
		1:5	2	Died after 4 days	" " " "
E	80	1:2	2	Alive	After 23 days following 5th injection
		1:5	2	"	" " " "
		1:10	2	Died after 4 days	" " " "
O	84	1:2	2	Alive	After 23 days following 4th injection
		1:5	2	Died after 8 days	" " " "
	Control		1	Died after 4 days	

These favorable results induced us still more to expand our research. We vaccinated 6 new testees. The doses of anatoxin used for the injections were approximately the same. The first two injections were 0.5 cm<sup>3</sup> each and the next injections were 1 cm<sup>3</sup> each. Thus, the subjects immunized 4 times received 3 cm<sup>3</sup> each and those immunized 5 times received 4.5 cm<sup>3</sup> of anatoxin each.

The last experiment indicates that sera of different people manifest diverse states (see Table 2). The serum of testee B positively held antitoxin, because the latter delayed up to 12th day the death of guinea pig after the latter received injection of a mixture of serum with 2 lethal doses of the toxin.

Still better results were obtained from sera of testees N and K in dilutions of 1:2 and 1:5 (N); the sera fully protected a guinea pig from two fatal doses of the toxin.

The serum of testee C, in dilution of 1:2, saved from death a guinea pig and, when diluted 1:5, it delayed up to 8th day the death of another guinea pig.

The sera of testees P and M showed no activity.

The results of these experiments verified our expectations. The vaccination with botulinum anatoxin performed on 40 testees proved harmless and even provided the immunization stimulation in several instances.

Many different trends taken by immunologists in their investigations usually revealed a very interesting fact that anatoxic vaccinations, when resumed after one year, lead to a development of immunity in a much shorter time and to a much higher degree.

Thus, comparing our old records and the latest results of RAMON'S experiment, we decided to resume the vaccination of our last year's volunteers. Some of them resumed the vaccination with willingness, although, at first, they disagreed with a plan of blood tests for examination of the state of serum one year after the interruption of immunisation.



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the 10. We vaccinated 9 testees with a single injection of 1 cm<sup>3</sup> of anatoxin. Then, 35 days later, blood specimens were taken from all

Table 3

Name	Age	Dilution of serum	Lethal doses of toxin	Results
P	70	1:2 1:5	3 3	Alive
B	68	1:2 1:5	3 3	"
YA	74	1:2 1:5	3 3	"
L	39	1:2 1:5	3 3	"
E	60	1:2 1:5	3 3	"
P	27	1:2 1:5	3 3	"
Control experiment	-	-	3	Died after 6 days
"	"	-	10	Died after 2 days

testees and the serum with the pertinent toxin was tested on guinea pigs (see Table 3).

The conclusion of this experiment suggests itself as follows: the serum of all vaccinated testees possessed antitoxin, which neutralized 3 fatal doses of the toxin, while serum's dilution was 1:2 and even 1:5.

The well-being of guinea pigs, which in some instances showed no reaction at all on administered mixture of toxin and serum, induced us to have confidence in good results even from higher dilutions of the serum.

In order to verify this theory, we conducted experiment with serum diluted 1:10 and 1:20 (see Table 4).

Table 4

Name	Age	Dilution of serum	Letthal doses of toxin	Results
L	39	1:10	3	Guinea pig alive
		1:20	3	" " "
Control experiment		-	3	Guinea pig died after 6 days
"	"	-	3	Guinea pig died after 6 days

The result was excellent. The serum used even in dilutions of 1:10 and 1:20 protected guinea pigs from 3 fatal doses of the toxin, while control guinea pigs died from the same dose.

#### Conclusions

1. Botulin anatoxin, accurately prepared, is absolutely harmless to man.
2. A multiple subcutaneous immunization of 40 testees with anatoxin caused neither general, nor local symptoms.
3. The immunization with anatoxin developed immunity to botulin in man.
4. The effectiveness of immunization depends on a dose of administered antitoxin.
5. The resumption of immunization (even a single administration) after one year resulted in a distinct increase of immunity to botulin.